

THE FLICKER RESPONSE CONTOURS FOR GENETICALLY  
RELATED FISHES. II\*

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(Accepted for publication, November 16, 1938)

I

A primary condition of rational physiological analysis is the discovery of organic invariants. An organic invariant is a property of the organism expressing its capacity to exhibit a particular kind of performance. The property clearly must be determinate,<sup>1</sup> in the sense that it is experimentally recoverable in adequate repetitions of the observations, else we have no right to regard it as expressing anything. The quantitative measure of this property should be independent of the specific magnitudes of the inciting conditions. Considerations of this sort are peculiarly important for the study of behavior,<sup>1</sup> although obviously not confined to this field, and for the use of data based upon organic response.

Response to visual flicker supplies an example.<sup>2</sup> The elementary problem is to deduce from the properties of the data of such responses the nature of the system in which these properties are determined and by which their exhibition is governed. This is essentially a problem of statistical mechanics. Its solution requires the use of

\* Part I is in *J. Gen. Physiol.*, 1937-38, **21**, 17-56. Some of the results have been discussed in a series of notes under the title "Specific constants for visual excitation," in *Proc. Nat. Acad. Sc.*, 1937, **23**, 516; 1938, **24**, 221, 542; 1939, **25**, 78.

We are under obligation to Dr. Gertrud Zerrahn-Wolf for assistance in connection with the experiments.

<sup>1</sup> Crozier, W. J., in Murchison, C., *The foundations of experimental psychology*, Clark University Press, Worcester, 1929, pp. 45-127. 1935, *Déterminisme et variabilité*, Paris, Hermann et Cie, 57 pp.

<sup>2</sup> Crozier, W. J., and Pincus, G., *J. Gen. Physiol.*, 1929-30, **13**, 57, 81; 1931-32, **15**, 437; 1936-37, **20**, 111.

those invariant indices which the data may be shown to provide. It cannot be directly resolved by qualitative appeal to structural features of the receptor mechanism, for several reasons: the data are basically derived from reactions of the whole organism, and are signals of marginal response to (recognition of) flicker; and the invariant general properties of the data, provided by the type of analytical function describing them, show no specific correlations with known details of structure in different animals.<sup>3</sup> The assumption that visual data are determined by quantitative properties of the retina is unnecessary and probably fallacious.<sup>4</sup> The parallelism with known retinal properties derives simply from the fact that a large number of (variable) elements are involved in each of the two cases; the invariants concerned are essentially statistical quantities.

It is obvious, however, that the flicker response curves for different kinds of animals are not the same. This means that if the same analytical function is usable for all, specific magnitudes must be exhibited by the parameters of the function. To demonstrate the descriptive validity of an interpretive function we require evidence (1) that the curve is in particular cases quantitatively repeatable, (2) that its specific parameters are definitely determined by the constitution of the organism, (3) that the proposed parameters have, experimentally, those physical properties called for by the interpretation.

For a considerable diversity of animals now tested, the law defining the relation between flash frequency ( $F$ ) and flash intensity ( $I$ ) critical for response to flicker is of the same general form.<sup>5</sup> It is of particular moment to supply proof that the *specific* properties of the curve, as distinct from its general character, are determined by the molecular constitution of the organism. This is an essential aspect of the proof that the descriptive invariant indices sought are *organic* invariants—properties of the material considered. This kind of proof can be provided by fortunately chosen cases open to genetic manipulation.

<sup>3</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Proc. Nat. Acad. Sc.*, 1937, **23**, 516; 1938, **24**, 125, *etc.*

<sup>4</sup> Cf. Crozier, W. J., and Holway, A. H., *J. Gen. Physiol.*, 1938–39, **22**, 341.

<sup>5</sup> *J. Gen. Physiol.*, 1937–38, **21**, 223, *etc.*

The logic of the situation is eminently simple.<sup>6</sup> For animals of a given type the curve of (mean) critical illumination ( $I_m$ ) as a function of flash frequency ( $F$ ) with flash cycle of a fixed type, and temperature constant, is a definitely recoverable function. The "curve" is of course a band, defined by  $I_m \pm \sigma_I$  as a function of  $F$ ,<sup>7</sup> describing the probability of occurrence of the index-response under the given conditions. The conditions of observation have been the same in all our experiments, and the variation involved is essentially a constitutional property<sup>8</sup> of the particular organisms concerned in the experiment. We need not for present purposes discuss how it may be supposed that this variation in excitability is implicated in, or dependent upon, the mechanism of response. We are concerned only, at the moment, with the fact of the reproducibility of the  $F - I_m$  curve, with material of the same type; and with the conjoined fact that the  $F - I_m$  curves for different types of animals, under the same conditions, are not the same.

The most useful analytical function adequately describing the dependence of  $I_m$  upon  $F$  (or of  $F_m$  upon  $I$ ) is a probability integral in  $\log I$ .<sup>9</sup> The equation for this curve accurately describes the course of the data in cases where only a single assemblage of sensory units is involved, uncomplicated by gross mechanical conditions;<sup>10</sup> and it permits precise dissection of the duplex flicker response contour typical of most vertebrates.<sup>11</sup> The three parameters of the probability curve (maximum, abscissa of inflection, and standard deviation of the first derivative) are severally influenced by temperature,<sup>12</sup> retinal area,<sup>13</sup> and fractional light time in the flash cycle<sup>14</sup> in ways which are

<sup>6</sup> *J. Gen. Physiol.*, 1929-30, **13**, 57; 1936-37, **20**, 111; 1937-38, **21**, 17.

<sup>7</sup> *J. Gen. Physiol.*, 1935-36, **19**, 503. *Proc. Nat. Acad. Sc.*, 1936, **22**, 412. *J. Gen. Physiol.*, 1936-37, **20**, 211, 363. *Proc. Nat. Acad. Sc.*, 1938, **24**, 130. *J. Gen. Physiol.*, 1937-38, **21**, 17, etc.

<sup>8</sup> *J. Gen. Physiol.*, 1936-37, **20**, 211, 363; 1937-38, **21**, 17.

<sup>9</sup> *Proc. Nat. Acad. Sc.*, 1937, **23**, 71, 516; 1938, **24**, 125, 216. *J. Gen. Physiol.*, 1938-39, **22**, 311, 451, in press.

<sup>10</sup> *J. Gen. Physiol.*, 1938-39, **22**, 311, 451, in press.

<sup>11</sup> *J. Gen. Physiol.*, 1937-38, **21**, 17, 203, 313. *J. Exp. Zool.*, 1939, in press.

<sup>12</sup> *J. Gen. Physiol.*, 1936-37, **20**, 393, 411. *Proc. Nat. Acad. Sc.*, 1938, **24**, 216.

<sup>13</sup> *J. Gen. Physiol.*, 1937-38, **21**, 223.

<sup>14</sup> *J. Gen. Physiol.*, 1937-38, **21**, 313, 463.

predictable by the conception of variable excitation units if these three parameters are acceptable as sufficient and efficient for the analytical function describing the data. This is sufficiently demonstrated by the fact that situations superficially dissimilar but in essential respects dynamically identical (*e.g.*, auditory excitation, nerve excitation; and photosynthesis) are describable by the same formulation.<sup>15</sup> No other available descriptive function adequately fits the measurements, although several have been proposed;<sup>16</sup> nor do the constants which these alternative descriptions contain exhibit the requisite physical properties which theory and experiment alike demand;<sup>17</sup> they therefore do not need to be considered.

The problem arises therefore as to whether the specific magnitudes of the parameters of the probability integral can by a genetic test of their constitutional dependence be shown to exhibit the behavior of natural constants. In the biological situation, and for the present problem in particular, this of course means *specific* constancy correlated with constitution. This can be tested by breeding experiments. For this purpose fresh water teleosts are peculiarly advantageous. Various genera which we have examined give different  $F - \log I_m$  curves. Several "species" and forms of each of several different generic types show agreements in this respect, as we have already found indicated.\* The constitutional differences involved in the control of the  $F - \log I$  curves therefore must be dependent upon rather deep-seated features of organization. Different genera, with certain limitations, can be successfully hybridized, as is well known.

We shall distinguish the three parameters of the probability function by symbols— $F_{max}$ , the maximum flash frequency to which the curve rises;  $\tau'$ , the ( $\log I$ ) abscissa of the inflection point; and  $\sigma'_{\log I}$ , the standard deviation of the first derivative of  $F$  vs.  $\log I$ ,

<sup>15</sup> *Proc. Nat. Acad. Sc.*, 1937, **23**, 71.

<sup>16</sup> Hecht, S., and Verrijp, C. D., *J. Gen. Physiol.*, 1933, **17**, 251. Hecht, S., Schlaer, S., and Smith, E. L., Intermittent light stimulation and the duplicity theory of vision, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1935, **3**, 237. Hecht, S., *Physiol. Rev.*, 1937, **17**, 239. Hecht, S., *The Harvey Lectures*, 1937–38, **33**, 35.

<sup>17</sup> *J. Gen. Physiol.*, 1936–37, **20**, 393, 411. *Proc. Nat. Acad. Sc.*, 1938, **24**, 216. *J. Gen. Physiol.*, 1937–38, **21**, 313, 463.

with  $F_{max.} = 100$  per cent. It is to be expected that in different forms  $\tau'$ , and  $F_{max.}$ , with temperature and  $t_L/t_D$  constant, may well be influenced by complex circumstances affecting (a) the total number of potentially excitable elements, and (b) the basic excitability of each (any) element. This is based upon the experimentally determined influence of temperature<sup>12</sup> and flash cycle light time fraction.<sup>14</sup> But  $\sigma'_{\log I}$  could be expected to be a more direct expression of the fundamental molecular organization of the animal, since it measures the relative dispersion of the  $\log I$  thresholds of the relevant population of neural elements; this quantity is experimentally independent of temperature<sup>12</sup> and of the light time proportion in the flash cycle,<sup>14</sup> and of retinal area,<sup>13</sup> but it is a specific property already indicated to be rather simply determined genetically.<sup>18</sup> (The situation may in this respect be complicated by certain purely mechanical factors among arthropods utilized for similar experiments,<sup>19</sup> but this does not really affect the immediate argument.)

In dealing with the flicker response contours of most vertebrates we encounter a situation which has a number of interesting features. Two populations of excitable elements are usually involved in the exhibition of the response to flicker, as revealed by the form of the flicker response contour. One of these has a modal excitability at a rather low critical intensity, the other (larger) has a modal excitability at a much higher intensity.<sup>20</sup> In terms of the duplexity theory these have been interpreted as due to the excitation of retinal rods and cones respectively. There are curious pitfalls in the logic of this proposition, into the discussion of which we need not now go. We are concerned only with the objective evidence that two populations of neural effects are concerned. In a genetic experiment with fishes<sup>18</sup> evidence has been provided that these two populations may in hereditary determination follow different rules. The properties of the flicker response contour in single types of individuals therefore provided important instances of the significance of the viewpoint here developed for the analysis of functional properties.

<sup>18</sup> *J. Gen. Physiol.*, 1937-38, **21**, 17. *Proc. Nat. Acad. Sc.*, 1937, **23**, 516.

<sup>19</sup> *J. Gen. Physiol.*, 1937-38, **21**, 223; 1938-39, **22**, 451.

<sup>20</sup> Cf. Hecht, S., *Physiol. Rev.*, 1937, **17**, 239. *J. Gen. Physiol.*, 1936-37. **20** 211; 1937-38, **21**, 17, 203. *J. Exp. Zool.*, 1939, in press.

Our first test of this matter<sup>18</sup> was exploratory. We used a back-cross stock derived from *Xiphophorus helleri* (*X.*)  $\times$  *Platypoecilus maculatus* (*P.*),  $F_1$ 's being mated to *X. helleri*. These individuals ( $H'$ ) were uniform in their flicker response contours (*i.e.*, showed no evidence of segregation into several types). There was indicated a fairly simple relationship between the specific values of the parameters of the hybrid curve and of the curves for the two parental stocks. The differences found between various genera of fishes have to do with  $F_{max.}$ ,  $\log I_{infl.}$  ( $= \tau'$ ), and  $\sigma'_{\log I}$ , for the "rod" and "cone" portions of each duplex curve, and with the separation between  $\tau'$  for the two parts. There is no evidence of formal association between magnitudes of these parameters in our data on various vertebrates, nor with respect to the rod and cone curves for any one kind of animal. (This refers, of course, to data obtained at one temperature, and with a flash cycle in which  $t_L = t_D$ ; we do not as yet know in sufficient detail just how the temperature characteristics for  $\tau'$ , and the proportionality factors for change of  $F_{max.}$  and  $\tau'$  with change of percentage light time, may also show specific association with genetic types.) In the breeding experiment<sup>18</sup> referred to it appeared that the cone  $F_{max.}$  in the hybrids was the same as for *X.*, and the values of  $\sigma'_{\log I}$  for both rod and cone curves; for the rod part, however,  $F_{max.}$  was the same as for *P.*;  $\tau'$  for both rods and cones was intermediate between the values for *X.* and *P.*, but closer to those for *P.*

Scrutiny of these findings shows that dominance with respect to one aspect of the flicker contour may be accompanied by "blending" with respect to another, and the dominance relations may be quite different in the two parts of the duplex curve. These are sufficient illustrations of the necessity for knowledge of the complete function before its rational interpretation and use become possible. Unquestionably many another genetic situation will in time be fruitfully explored from this standpoint.

It is also clearly indicated that the parameters  $F_{max.}$  and particularly  $\sigma'_{\log I}$  behave as if determined by some relatively simple fact of organization. The presumption is encouraged that a determinate physical basis exists of which they are expressions. They refer respectively to the total number of excitation elements, given by

$\int_{-\infty}^{+\infty} dF/d \log I$ , and to the frequency distribution of the  $\log I$  thresholds. The resistance of  $\sigma'_{\log I}$  to experimental conditions, shown in its constancy with respect to area, temperature, and fractional light time in the flash cycle, is greater than that of  $F_{max.}$ , which (although independent of temperature) is in simple proportion to the percentage dark time in the flash cycle. It is, of course, probable that the proportionality factor in this relationship would also prove to be a specific characteristic. Conditions might easily be conceived in which the value of  $F_{max.}$  (for a given ratio  $t_L/t_D$ ) could be modified by circumstances affecting the excitability cycle of each irritable element; this is in line with the explanation<sup>14</sup> for the direct proportionality of  $F_{max.}$  to  $t_D/(t_L + t_D)$ . Hence the genetic behavior of  $F_{max.}$  might well be expected to be less simple than that of  $\sigma'_{\log I}$ . The same applies to the abscissa of inflection,  $\tau'$  or  $\log I_i$ . The dependence of  $\tau'$  upon temperature and  $t_L/t_D$  is simple, and of such a sort as to show that conditions proportionately affecting the excitabilities of all the elements in the same way naturally shift the  $F - \log I$  curve to a new position on the  $\log I$  scale, without changing  $F_{max.}$ . Any number of circumstances involving the chemical control of the individual excitabilities, or even conditions modifying the mere optical transmission of the ocular mechanism, could be expected to modify  $\tau'$ . The question as to whether the temperature characteristics for the shift of  $\tau'$  are specific, and heritable,<sup>21</sup> presents another aspect of this matter which will ultimately require investigation, but concerning which we have as yet no information; the corresponding question as to the quantitative dependence of  $\tau'$  upon  $t_L/t_D$  is likewise unexplored.

In developing an interpretive theory of the flicker response contour there are to be recognized two distinct aspects:

(a) The proof that the shape of the curve is dependent upon the constitutional organization of the animal is supplied by the facts of reproducibility under fixed conditions, differences with different types of animals, invariance under alteration of environmental factors, and particularly its behavior in inheritance. This provides the conditions under which it becomes possible to undertake a de-

<sup>21</sup> For an analogous instance, cf. Pincus, G., *J. Gen. Physiol.*, 1930-31, **14**, 421.

termination of the properties of the curve as revealed by the relations of these properties to experimentally controllable variables.

(b) It does not select for us the particular analytical function most appropriate for the data. It does, however, supply explicit criteria which the chosen function must satisfy. The results of this first experiment, as we indicated, were surprising—since no definite, particular expectations of simplicity in the result could have been reasonably entertained. A second experiment of this type was therefore planned, under somewhat simpler conditions. The  $F_1$  progeny of a cross between *Xiphophorus* and *Platypoecilus* was to be examined, and the  $F_1$ 's interbred. We sought to utilize if possible species of *X.* and *P.* different from those involved in the earlier test. For assistance in obtaining stocks of the desired animals we are under obligation to our associate Dr. C. P. Haskins. This paper is based on the data secured with *X.*, *P.*, and their  $F_1$  offspring. The results with  $F_2$  will be considered in a subsequent report.

## II

The observational procedure used has been kept as uniform as possible. Groups of individuals are well adapted to routine conditions of feeding and experimental handling. Where possible a lot of 10 is employed. In certain cases a smaller set must be used as no more fishes of that particular type are available. Records are kept for each individual separately. Three successive observations of critical illumination are made on each individual, at each flash frequency. The individual means ( $I_1$ ) of these sets of three are averaged for the group, and this appears as  $I_m$  in the tables. This is done only if analysis of the data shows that the individuals in a group are equivalent. The reasons for this averaging have been considered previously,<sup>18</sup> and the tests for equivalence (homogeneity). The readings of critical illumination are amply sensitive to detect real individual differences even when these are quite small.<sup>22</sup> The succession of the levels of  $F$  tested is so arranged as to reveal drifts of  $I_m$  with time during the completion of a curve, should these occur.

<sup>22</sup> *J. Gen. Physiol.*, 1936-37, **20**, 363, 393; 1937-38, **21**, 223; 1938-39, **22**, 311. *J. Exp. Zool.*, 1939, in press.

The apparatus has been described.<sup>18</sup> Temperature was maintained at 21.5°. The flash cycle used had equally long intervals of light and no light.

The data on *X. montezuma* are given in Table I. It is apparent from comparison with the measurements with *X. helleri*<sup>18</sup> at the same flash frequencies that the agreement between the results with these

TABLE I

Showing the essential similarity of the flicker response contours for two species of *Xiphophorus*. Mean critical intensities with the P.E.'s of their dispersions (millilamberts). Temperature 21.5°C., flash cycle with equally long light and dark intervals. The data on *X. helleri* are taken from Crozier, Wolf, and Zerrahn-Wolf, *J. Gen. Physiol.*, 1937-38, **21**, 25; only data at the indicated flash frequencies are repeated here; the complete set of measurements is given in Fig. 1 (together with data on albino individuals<sup>23</sup>). There is no significant difference between the measurements for *X. montezuma* and *X. helleri*. Three observations on each of the same 10 individuals in all cases.

F per sec.	<i>Xiphophorus montezuma</i>		<i>Xiphophorus helleri</i>	
	log $I_m$	log P.E. $\cdot I_1$	log $I_m$	log P.E. $\cdot I_1$
3	$\bar{6}.3528$	$\bar{7}.0030$	$\bar{6}.3420$	$\bar{7}.0969$
6	$\bar{5}.3354$	$\bar{7}.9304$	$\bar{5}.3721$	$\bar{7}.9653$
			$\bar{5}.2826$	$\bar{7}.7148$
			$\bar{5}.3539$	$\bar{7}.8341$
9	$\bar{3}.2393$	$\bar{5}.7749$	$\bar{3}.2469$	$\bar{5}.8295$
			$\bar{3}.2378$	$\bar{5}.8374$
15	$\bar{2}.8650$	$\bar{3}.4494$	$\bar{2}.8918$	$\bar{3}.2650$
25	$\bar{1}.4639$	$\bar{2}.1501$	$\bar{1}.4708$	$\bar{3}.9719$
			$\bar{1}.4598$	$\bar{3}.9677$
35	$0.1772$	$\bar{2}.7698$	$0.1520$	$\bar{2}.9002$
42	$1.3105$	$\bar{1}.7176$	$1.3057$	$\bar{1}.5398$
			$1.2785$	$\bar{1}.5864$

two superficially quite different animals is remarkably close. We have already shown<sup>23</sup> that albino mutants of *X. helleri* give figures not distinguishably different from those gotten with the wild type *X. helleri*. The determinations with the three forms of *Xiphophorus* are plotted together in Fig. 1.

<sup>23</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 221.

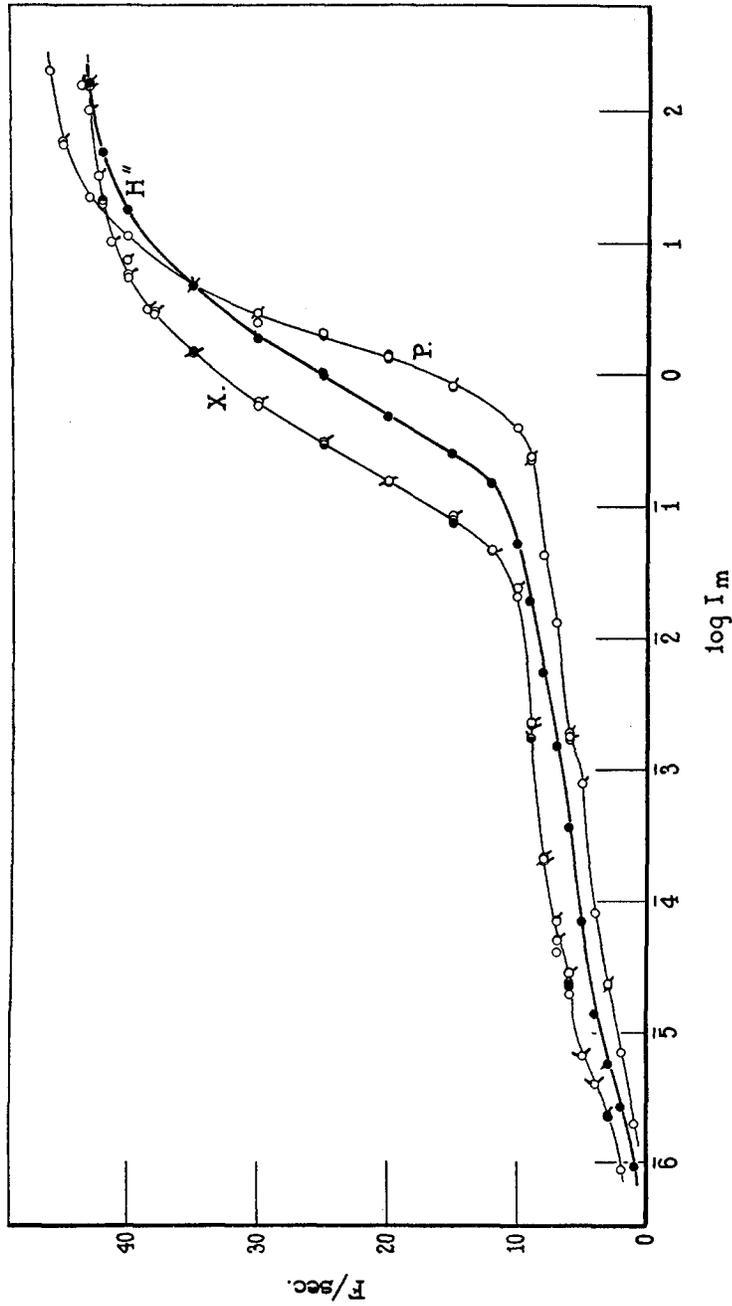


FIG. 1. Mean critical intensities as a function of flash frequency ( $f^{\circ} = 21.5; t_L = t_D$ ) for (X.). Several types of *Xiphophorus* (● = *X. montezuma*; ○ = *X. helleri*, with duplicating points as ○) for which data are given in Table I; and Q = *X. helleri* var. *albino*<sup>23</sup>; P. = several types of *Platypoecilus* (Table II; ○ = *P. maculatus*, Q = *P. variatus*); H'',  $F_1$  hybrids from *X. montezuma* × *P. variatus*, data in Table III.

Similar agreement of measurements with five different forms of *Platypoecilus*—including one species hybrid—is shown in Table II (Fig. 1).

These data agree with those previously secured<sup>18</sup> in showing the essential invariance of the flicker response contour under fixed conditions of temperature and light time fraction, over a considerable interval of time, for each of the generic types. The difference between the two curves, on the other hand, is striking and complex (cf. Fig. 1). The variation of  $I_1$  follows the usual rule for verte-

TABLE II

Showing the essential quantitative agreement between the flicker response contours for various forms of *Platypoecilus*: *P. maculatus nigra* (10 individuals); *P. variatus* (red tail) (3 individuals); *P. v. nigra* (2 individuals); *P. v. rubra* (2 individuals); *P. v., gold* (5 individuals); hybrids (2 individuals) between *P. variatus rubra* and *P. maculatus*. Three observations on each individual at all points. Temperature 21.5°C., flash cycle with 50 per cent light time. The variation indices are plotted in Fig. 2.

<i>F</i> per sec.	Red tail <i>P. variatus</i> log $I_m$	<i>P. v. nigra</i> log $I_m$	<i>P. v. rubra</i> log $I_m$	Gold platy <i>P. variatus</i> log $I_m$	Hybrids <i>P. v. rubra</i> × <i>P. maculatus</i> log $I_m$
3	$\bar{5}.3583$			$\bar{5}.3766$	$\bar{5}.3692$
6	$\bar{3}.2430$ ( $\bar{3}.2725$ )	$\bar{3}.2480$	$\bar{3}.2721$	$\bar{3}.2290$	$\bar{3}.2534$
9	$\bar{1}.3526$ ( $\bar{1}.3608$ )	$\bar{1}.3535$	$\bar{1}.3699$	$\bar{1}.3596$	$\bar{1}.3670$
15	$\bar{1}.8980$			$\bar{1}.8846$	$\bar{1}.9135$
25	$0.3002$			$0.2833$	$\bar{1}.3107$
35	$0.6801$			$0.6544$	$0.6815$
45	$1.7327$			$1.7276$	$1.7582$

brates with duplex flicker contours, in that  $P.E._{I_1}$  is directly proportional to  $I_m$  but beyond a certain intensity the proportionality constant changes.<sup>24</sup> This seems to be due to the complete dropping out of effects due to the rod population of elements above this particular intensity.<sup>24</sup> For these genera the break in the variation function (Fig. 2) is at comparatively high flash intensity (ca. antilog 0.5), so that only a small part of the data fall above this level. The mean

<sup>24</sup> *J. Gen. Physiol.*, 1935-36, **19**, 503; 1936-37, **20**, 211; 1937-38, **21**, 17, 203. *J. Exp. Zool.*, 1939, in press.

variation with  $P$ : is somewhat, and significantly, lower than with  $X$ . at the same level of  $I_m$ —for the intensities below antilog 0.5 in the ratio of 1:1.41. Analyses of the  $X$ . and  $P$ . duplex curves have already been given in some detail.<sup>18</sup> By extrapolation of the cone curve toward  $F = 0$  it is found that the “bump” on the nearly hori-

TABLE III

Mean critical intensities for response to visual flicker, with the P.E. of their dispersions (millilamberts) at various flash cycles for  $F_1$  hybrids between *X. montezuma* and *P. variatus*; three observations on each of the same 3 individuals at all points. Temperature 21.5°C., flash cycle with 50 per cent light time. See Figs. 1 and 2.

$F$	$\log I_m$	$\log P.E. \cdot I_1$
1	$\bar{7}.9561$	$\bar{8}.2335$
2	$\bar{6}.4140$	$\bar{8}.3892$
3	$\bar{6}.7543$	$\bar{8}.8189$
	$\bar{6}.7487$	$\bar{8}.3705$
	$\bar{6}.9534$	$\bar{8}.8235$
4	$\bar{5}.1348$	$\bar{7}.4622$
5	$\bar{5}.8390$	$\bar{7}.3373$
6	$\bar{4}.5544$	$\bar{6}.8235$
7	$\bar{3}.1708$	$\bar{5}.4355$
8	$\bar{3}.7273$	$\bar{4}.2953$
9	$\bar{2}.2730$	$\bar{4}.8475$
10	$\bar{2}.7118$	$\bar{3}.0759$
12	$\bar{1}.1706$	$\bar{3}.8098$
15	$\bar{1}.3993$	$\bar{3}.5833$
20	$\bar{1}.6701$	$\bar{2}.0795$
25	$\bar{1}.9880$	$\bar{2}.5750$
	$0.0020$	$\bar{3}.9943$
30	$0.2596$	$\bar{2}.9016$
35	$0.6767$	$\bar{2}.6062$
	$0.6751$	$\bar{2}.9928$
40	$1.2370$	$\bar{1}.0430$
42	$1.6761$	$\bar{1}.7760$
43	$2.2063$	$0.2100$

zontal intermediate portion of the curve (*cf.* Fig. 1) coincides with the start of the cone curve. For this extrapolation a log logistic may be used,<sup>18</sup> but a probability integral<sup>18</sup> is better. Cases in which the whole course of the curve may be followed for a single uncomplicated population of sensory effects<sup>9</sup> permit a decision between these

two equations. The greater separation of duplicate estimations of  $\log I_m$  in the region of beginning of the cone curve as compared with

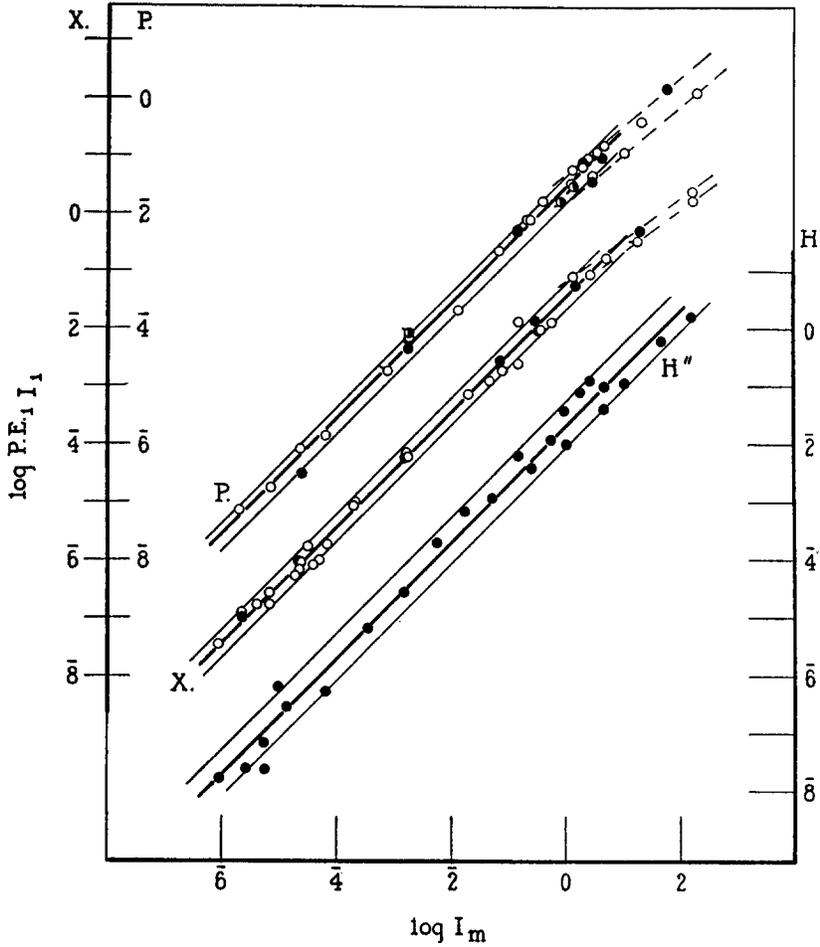


FIG. 2. The relation between dispersions of the measurements of critical intensity and the means, for *Platypoecilus* (P.),  $\circ$  = *P. maculatus*,  $\bullet$  = *P. variatus*, *Xiphophorus* (X.),  $\circ$  = *X. helleri*;  $\bullet$  = *X. montezuma*; and the  $F_1$  hybrids (H). Data in Table I.

other regions of the curve<sup>18</sup> is again confirmed by the new measurements.

Three  $F_1$  hybrids ( $H''$ ) between *X. montezuma* and *P. variatus* were used. They were entirely equivalent. Observations were obtained at nineteen flash frequencies. The data are summarized in Table III (see Fig. 1). The variation data (Fig. 2) show mean values of  $P.E._{I_1}$  a little lower than for *X.* and *P.*, but this is probably a simple consequence of the smaller number of individuals (3) and of

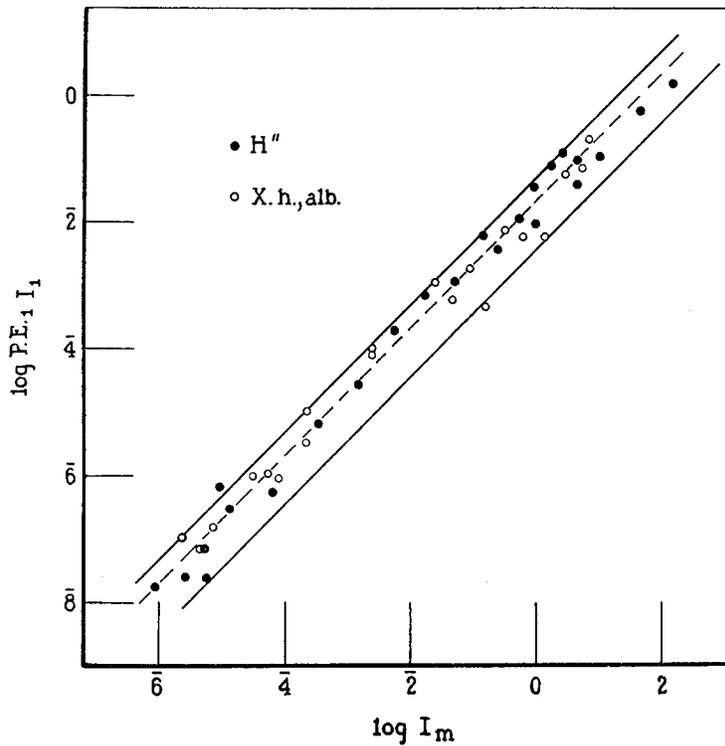


FIG. 3. The variation of  $I_1$  follows the same rules for the  $F_1$  hybrids and for a set of the same number of individuals (three) of *X. helleri* var. *albino*. See text.

readings (9) at each point. The variation data for  $H''$  indeed agree precisely with those from *X. helleri* var. *alb.*,<sup>18</sup> where the same numbers were used (Fig. 3).

The behavior of these fishes exhibits certain minor differences with respect to the elicitation of the index response.<sup>18</sup> *X. montezuma*, like *helleri*, is very active and is liable to disturbance by vibrations.

The response to flicker consists in the beginning of swimming in the direction of motion of the revolving stripes. This may be quickly stopped so that the fish is quite stationary but with rapid fin undulations. Males especially may also show a quick shaking (rolling) motion at the threshold of the response. The reactions used are only really clear when the fish is near the wall of the container and headed in the direction of revolution of the stripes. These conditions sometimes must be patiently waited for. At low  $F$ 's (below 10) the response is as clear as the movement of a galvanometer needle. With higher  $F$ 's the tendency to rigidity is more pronounced. In these matters the hybrids resemble  $X.$ , and not  $P.$  (*cf.* footnote 18), but the shaking movements are less in evidence.

### III

The data of Tables I, II, and III, are plotted in Fig. 1. Analysis of the  $H''$  curve by the method already used with other forms<sup>18</sup> exhibits (Fig. 4) the dissection of the duplex graph into a lower portion (rod) and an upper (cone) part with a region of overlap in which the effectiveness of the rod contribution progressively declines. The dissection is made by extrapolation of the probability integral adjusted to the upper portion of the  $H''$  data in Fig. 1. The declining rod curve is obtained by difference. The reasons for treating  $F$  as additive have been considered previously.<sup>25</sup>

The uncomplicated cone portions of the flicker contour for  $X.$  and  $H''$  are given upon a probability grid in Fig. 5. The data upon the backcross hybrid  $H'$  of  $X. helleri$  and  $P. maculatus$  previously studied<sup>18</sup> are also included. A satisfactory fit is obtained by finding the value of  $F_{max}$  which makes the graph (Fig. 5) rectilinear. For  $H'$  and  $X.$ ,  $F_{max}$  is essentially identical, respectively 43.00 and 43.05/sec. For  $H''$  a slightly higher value is required, 43.34. But the slope is the same for all three. This of course means that the cone  $\sigma'_{log I}$  is identical. It was shown previously<sup>18</sup> that for  $P.$   $F_{max}$  is of course much higher and  $\sigma'_{log I}$  much less.

The abscissa of inflection for  $H'$  and  $H''$  is intermediate between those for  $X.$  and  $P.$ , that for  $H''$  less ( $I_i = 0.576$ ) than for  $H'$  ( $I_i = 0.832$ ).

<sup>25</sup> *J. Gen. Physiol.*, 1936-37, 20, 411.

It has been pointed out that  $F_{max}$  could well be influenced by factors tending to increase the effective size of the population of sensory elements. *X. montezuma* is larger than either *X. helleri* or the hybrids, but there are no differences in any of the fishes we have tested to be correlated with individual differences of size. The effect is probably of a more subtle sort, like that induced by a slight shortening of the light time interval, as already suggested (section I). The agreements of  $\sigma'_{\log I}$  are definite and striking. They must be taken as evidence for the dominance of *X.* type of cone population over that seen in *P.* The shift of  $\tau'$ , different in the two kinds of hybrids considered, is exactly of the sort produced by a lowering

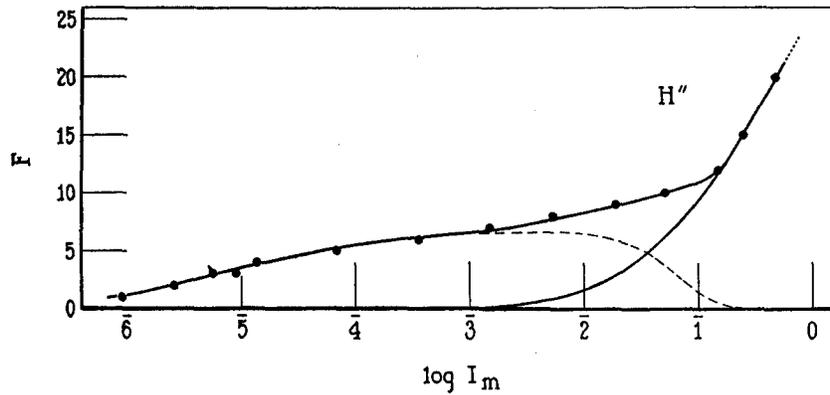


FIG. 4. Analytical dissection of the duplex flicker contour for  $H''$  by extrapolation of the probability integral which describes the upper segment of the graph (Figs. 1 and 5). See text.

of the temperature.<sup>12</sup> It clearly corresponds to the effect predictable if in the hybrids the effective concentration of material governing excitability were to be reduced, so that with temperature fixed the rate of chemical transformations for which it is responsible is lowered—therefore a higher intensity of light is required for response at a fixed  $F$ . (The reasons leading to this type of interpretation have been gone into at some length in other connections<sup>26</sup> and do not require restatement here.) The effect cannot be interpreted as due

<sup>26</sup> *J. Gen. Physiol.*, 1938-39, 22, 311, 487.

to a difference in the transparency of the optic media, since the shift for rod and cone segments is not the same. (No iris movements are involved either.) It would be expected on this basis, that the temperature characteristics for the cone curves  $X.$ ,  $H'$ , and  $H''$  should

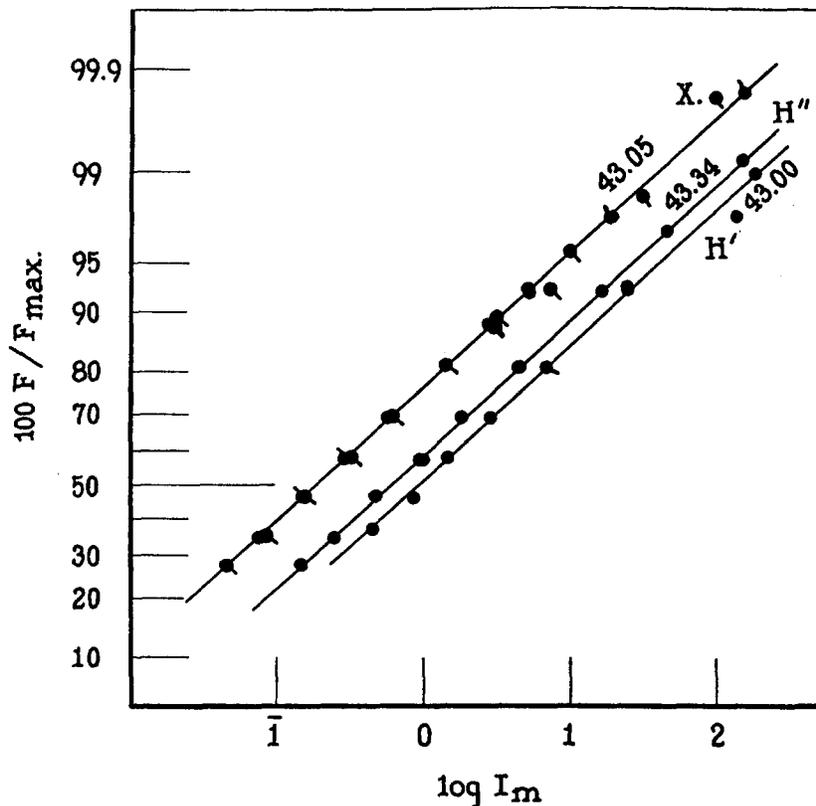


FIG. 5. The upper segments of the flicker contours for *Xiphophorus* and two kinds of its hybrids with *Platypoecilus* on a probability grid. The slopes are identical (*i.e.*,  $\sigma'_{\log I}$  is the same). The curves are computed to maxima 43.05 ( $X.$ ), 43.34 ( $H''$ ), and 43.00 ( $H'$ ).

be the same, although this might be difficult to establish if two temperature characteristics are exhibited over the explorable range of temperatures.<sup>26</sup> The rôle of such considerations in suggesting experimental tests of the mechanism invoked to account for the phenom-

ena connected with critical temperatures<sup>26</sup> is not to be lost sight of, and may easily prove to be quite important.

The outcome of the analysis, as regards the cone section of the graph, is to make it fairly evident that slight, quantitative modifications, expressed through the activity of essentially the same kind of population of sensitive elements, with the same type of physico-chemical architecture, must be conceived to determine the form of the upper (cone) segment of the flicker response contour in *Xiphophorus* and in its hybrids with *Platypoecilus*. The shape of the curve provides an elementary instance of classical hereditary dominance. It is obvious that from the standpoint customarily used in the majority of genetic analyses this phenomenon could not be recognized (*cf.* also footnote 27). It is also clear that with respect to the same kind of organic performance two quite different sorts of hereditary influence are simultaneously manifest:  $\tau'$  supplies an example of "blending,"  $\sigma'_{log I}$  of simple dominance. This serves as another<sup>27</sup> instance of the way in which situations may be totally obscured in the absence of knowledge of the entire course of the performance contour. The case becomes even more curious and illuminating when the lower (rod) part of the curve is carefully examined.

For  $H'$  it was shown that in the rod region  $\tau'$  is also intermediate between that for  $X$ . and for  $P$ . This is likewise true for  $H''$  (Fig. 6).  $F_{max.}$  (rods) for  $H'$  was, however, equivalent to that for  $P$ . although  $\sigma'_{log I}$  was that of  $X$ . With  $H''$  it is apparent that  $F_{max.}$  is exactly intermediate between those for  $X$ . and for  $P$ ., but  $\sigma'_{log I}$  is identical with that for  $P$ . (Fig. 6).

The fuller interpretation of these facts depends in great measure on the outcome of tests made with  $F_2$  and backcross stocks which (with certain limitations due to the genetic nature of generic crosses) should give evidence as to possible segregation phenomena. This information we expect to be able to provide in the near future. It is made reasonably evident, however, that there are indeed involved in the flicker contour the properties of two distinct groups (populations) of sensory effects.<sup>28</sup> Whether these two are to be properly correlated in a direct way with the attributes of retinal rods and cones

<sup>27</sup> *Cf. J. Gen. Physiol.*, 1936-37, **20**, 111.

<sup>28</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 125. *J. Gen. Physiol.*, 1937-38, **21**, 17.

is a question beset with perplexities of a kind usually overlooked, and upon which we need not for present purposes elaborate. The two groups of effects are manifestly governed by different processes, since their determinants do not obey the same rules in inheritance. This is perfectly consistent with the fact that the relations of the rod and cone segments to temperature are not quite the same, in the one case thus far investigated;<sup>29</sup> the fact that the temperature characteristics<sup>29</sup> and the effects of supranormal temperatures<sup>30</sup> are the same for both merely shows that the character of the essential physico-

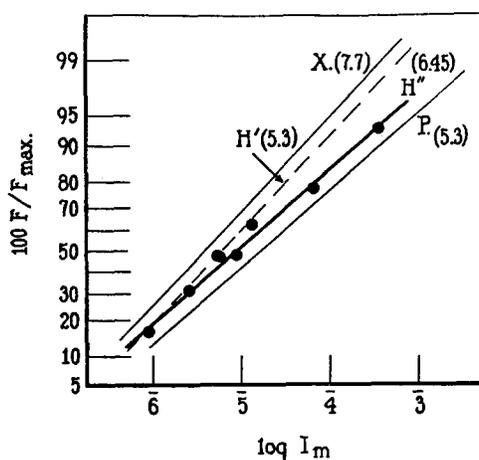


FIG. 6. The initial rising segment of the flicker response contour for the  $F_1$  hybrids ( $H''$ ) produced by  $X. \times P.$  has the slope constant ( $\sigma'_{\log I}$ ) of  $P.$ ; the cross-bred progeny  $H'$ , produced in another type of experiment, have  $\sigma'_{\log I}$  equivalent to that for  $X.$  The respective values of  $F_{max.}$  are:  $X.$ , 7.7;  $P.$ , 5.3;  $H'$ , 5.3;  $H''$ , 6.45. See text. The lines for  $X.$ ,  $P.$ , and  $H'$  are taken from the analyses previously published.<sup>18</sup>

chemical organization in the two sets of elements is the same;<sup>31</sup> it does not imply that the amounts of governing substances in the excitability systems of the two sets are identical. The differences

<sup>29</sup> *J. Gen. Physiol.*, 1938-39, **22**, 487.

<sup>30</sup> *J. Exp. Zool.*, 1939, in press.

<sup>31</sup> Also shown by the fact that in the same species the proportionality constants for enlargement of the cone and rod segments of the curve as the light time fraction in the flash cycle is increased, are identical: *J. Gen. Physiol.*, 1937-38, **21**, 313.

in the behavior of rod and cone sections of the curve with respect to dominance relations, as in  $H'' \sigma'_{log I}$  for one follows  $P$ . and the other the  $X$ . parent, complement the state of affairs in  $H'$ , where each adheres to the  $X$ . type, and rounds out the proof that the hereditary determination of the rod section is independent of that for the cone part.

## IV

The curve obtained by subtraction of the backward extrapolation of the cone probability integral from the composite flicker contour yields the difference curve<sup>18</sup> held to depict the way in which the dropping out of the rod contribution depends upon intensity as the excitation of the cone population increases. Fig. 6 has given this curve for  $H''$ . The statistical conception of the excitation elements required for the analysis<sup>22</sup> suggests that this curve should also be described by a probability integral. This has been demonstrated in various instances.<sup>11</sup> Fig. 7 shows that the same principle applies for the data on  $H''$ .

There are several ways in which the causation of this declining rod contribution could be interpreted. It could be considered as an intrinsic property of the rod population of neural effects.<sup>18</sup> In this case it might be reasonable to expect that if the cone population could be in some manner obliterated or suppressed the declining rod curve could then be directly determined by experiment. The measurement of the flicker contour for a nocturnal animal (gecko) possessing only rods<sup>23</sup> definitely shows that retinal rods are not necessarily restricted to low intensity functioning. For human subjects the evidence from day-blind, color-blind subjects<sup>24</sup> is, of course, defective, as one does not know except by circular argument that the retina there possesses only rods—or that if it does these are the equivalents of the rods in a normal retina (in fact, the logic of the situation suggests that, if the argument concerned be accepted, they cannot be such equivalents).

<sup>18</sup> *Proc. Nat. Acad. Sc.*, 1936, **22**, 412. *J. Gen. Physiol.*, 1937-38, **21**, 17, etc.

<sup>22</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 538. *J. Gen. Physiol.*, 1938-39, **22**, in press.

<sup>24</sup> Hecht, S., Shlaer, S., Smith, E. L., Haig, C., and Peskin, J. C., 1938, *Am. J. Physiol.*, **123**, 94.

Another possibility is that, in the duplex flicker curve, the excitation of cone elements neurally inhibits the influence of rod units. This can be indirectly tested by examining the morphology of the declining rod curve as it is deduced for various animals in which the relative magnitudes, positions, and shapes of the rod and cone segments of the duplex flicker contour are diversely differentiated. The suggestive fact is brought out by such an examination that the more sharply the cone curve rises, the more abruptly does the rod contri-

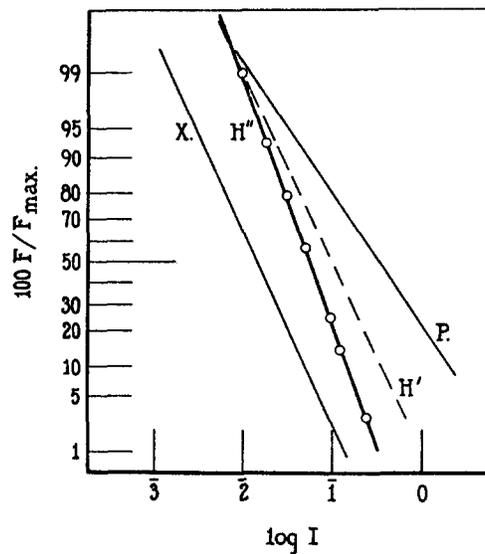


FIG. 7. Curves taken to measure the dropping out of rod contributions with increase of  $F$  are obtained by the method of dissection illustrated in Fig. 4. They adhere to probability integrals.<sup>18</sup> The points for  $H''$  are read from the curve in Fig. 4. Discussed in the text.

bution decline.<sup>24</sup> If the location of the declining rod curve, most easily estimated by its  $\log I$  of inflection, moreover is more extensively removed from the rising branch of the rod curve the wider the separation of  $\tau'$  for rods from that for cones,<sup>24</sup> and its rate of decline is therewith found lessened. These relations are only slightly obscured by the presence of relatively enlarged rod populations.

The present data supply a rather significant test of this view, in

a fairly homogeneous group of cases. As Fig. 7 shows,<sup>18</sup>  $\sigma'_{\log I}$  for *X.* and for *P.* are quite different. The declining rod curve for *X.* falls off much more steeply, and at the same rate as for *H'*. The *P.* cone curve rises more rapidly, but its rod population is smaller and more widely removed from the cone population. The *H'* rod curve is like the *X.* in shape, and the *H'* rod and cone curves are shifted (as compared with *X.*) by about the same extent on the  $\log I$  axis. With *H''*, however, we find a rate of decline of the rod contribution which is much higher than for *X.* and *H'*. In correlation with this, it is to be noticed that with *H''*  $\tau'$  for cones is less widely removed from that for rods than is true with *X.* or *H'*, or than in *P.*, while the rod population is smaller than in *X.* The data are therefore consistent and suggest pretty plainly that if by some means the cone effects could be suppressed we would find no dropping out of the rod contribution (*cf.* footnote 34); the pure rod curve of the gecko<sup>33</sup> shows no decline over the whole range of intensities open to test.

It is to be noted that these indications are entirely consonant with the conception that the quantitative properties of visual data, and specifically the data of flicker, are not determined by properties of the retina but measure on the contrary properties of central nervous events.

## V

## SUMMARY

The flicker response contour has been determined for several species and types of the teleosts *Xiphophorus* (*X.*) and *Platypoecilus* (*P.*) under the same conditions. The curve ( $F$  vs.  $\log I_m$ ) is the same for representatives of each generic type, but is different for the two genera. Its duplex nature is analyzable in each instance by application of the probability integral equation to the rod and cone constituent parts. The parameters of this function provide rational measures of invariant properties of the curves, which have specific values according to the genetic constitution of the animal. The  $F_1$  hybrids (*H''*) of *X. montezuma*  $\times$  *P. variatus* show dominance of the *X.* properties with respect to cone  $F_{max}$ . and  $\sigma'_{\log I}$ , but an intermediate value of the abscissa of inflection ( $\tau'$ ). The rod segment shows dominance of  $\sigma'_{\log I}$  from *P.*, but an intermediate value

of  $F_{max}$ , and of  $\tau'$ . The composite flicker curve involves the operation of two distinct assemblages of excitable elements, differing quantitatively but not qualitatively in physicochemical organization, probably only secondarily related to the histological differentiation of rods and cones because almost certainly of central nervous locus, but following different rules in hereditary determination, and therefore necessarily different in physical organization. The interpretation of the diverse behavior of the three parameters of the probability summation is discussed, particularly in relation to the physical significance of these parameters as revealed by their quantitative relations to temperature, retinal area, and light time fraction in the flash cycle, and to their interrelations in producing the decline of rod effects at higher intensities.

It is stressed that in general the properties of the parameters of a chosen interpretive analytical function must be shown experimentally to possess the physical properties implied by the equation selected before the equation can be regarded as describing those invariant properties of the organic system concerned upon which alone can deduction of the nature of the system proceed. The importance of genetic procedures in furthering demonstration that the biological performance considered in any particular case exhibits constitutionally invariant features provides a potentially powerful instrument in such rational analysis.